

533895

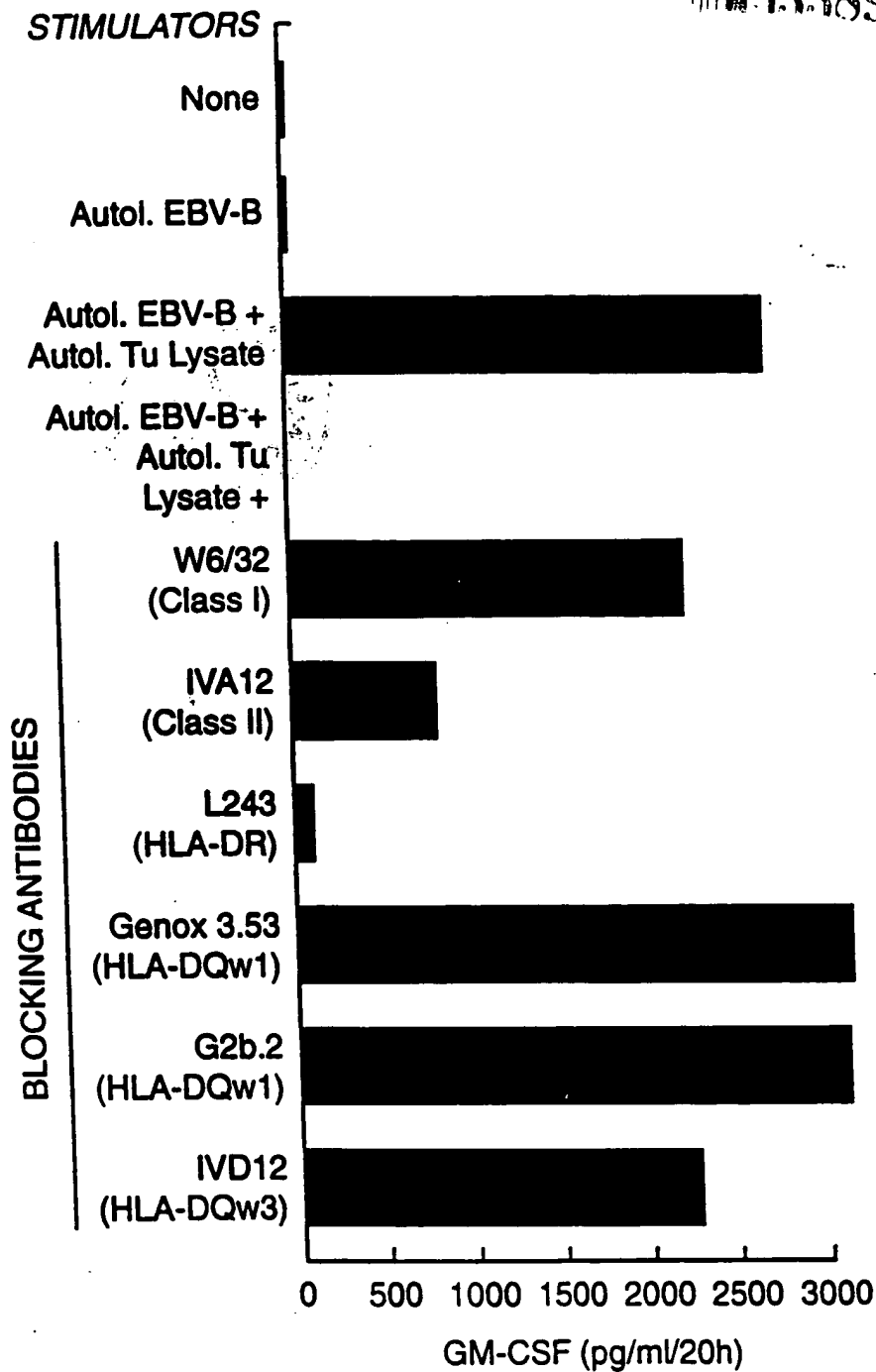


FIGURE 1

532895

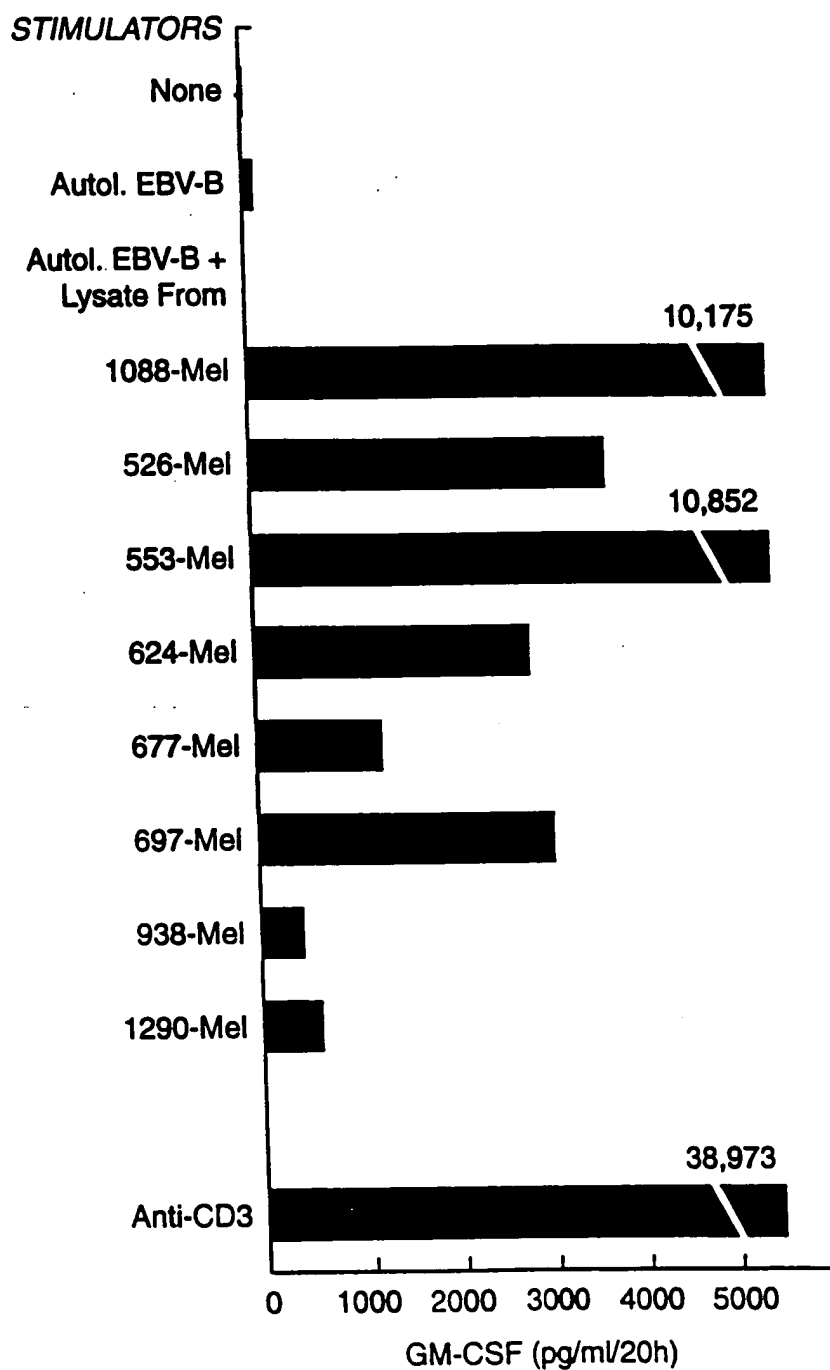


FIGURE 2

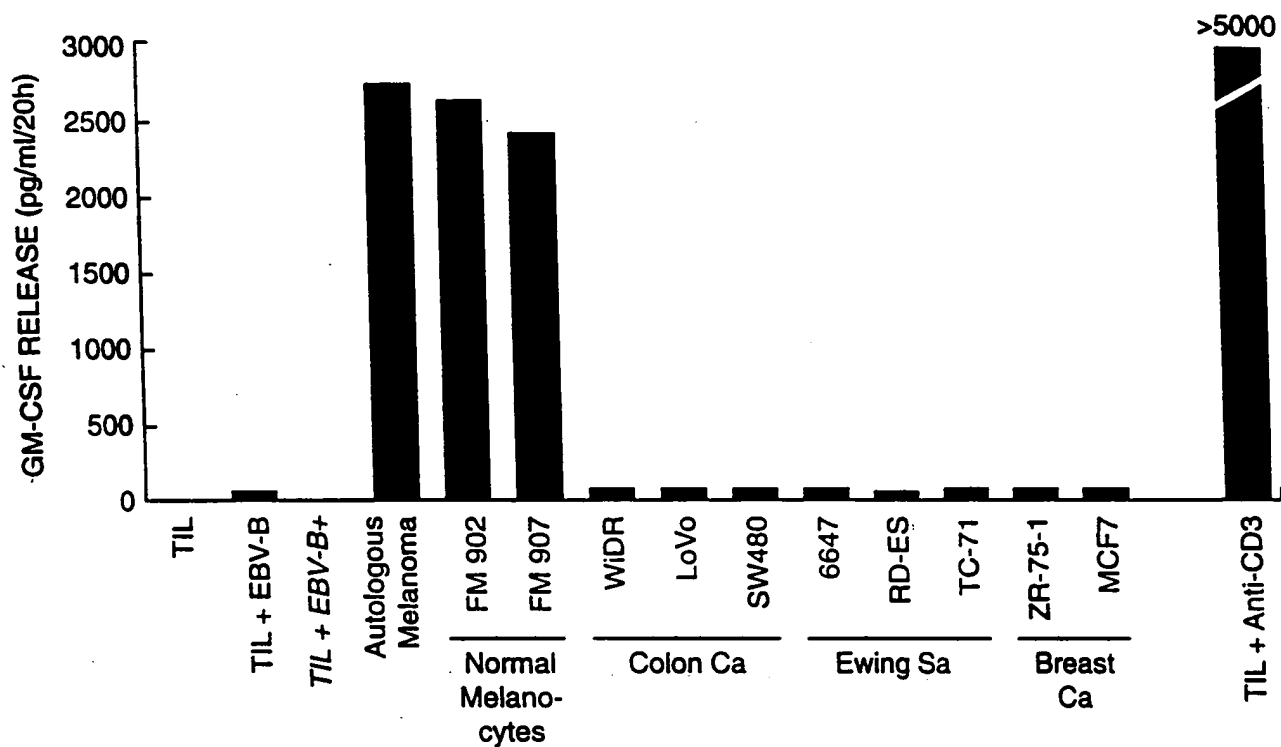


FIGURE 3

CD4+ TIL 1088 RECOGNIZE TY 56-70 AND TY 448-462 PRESENTED BY HLA-DR4

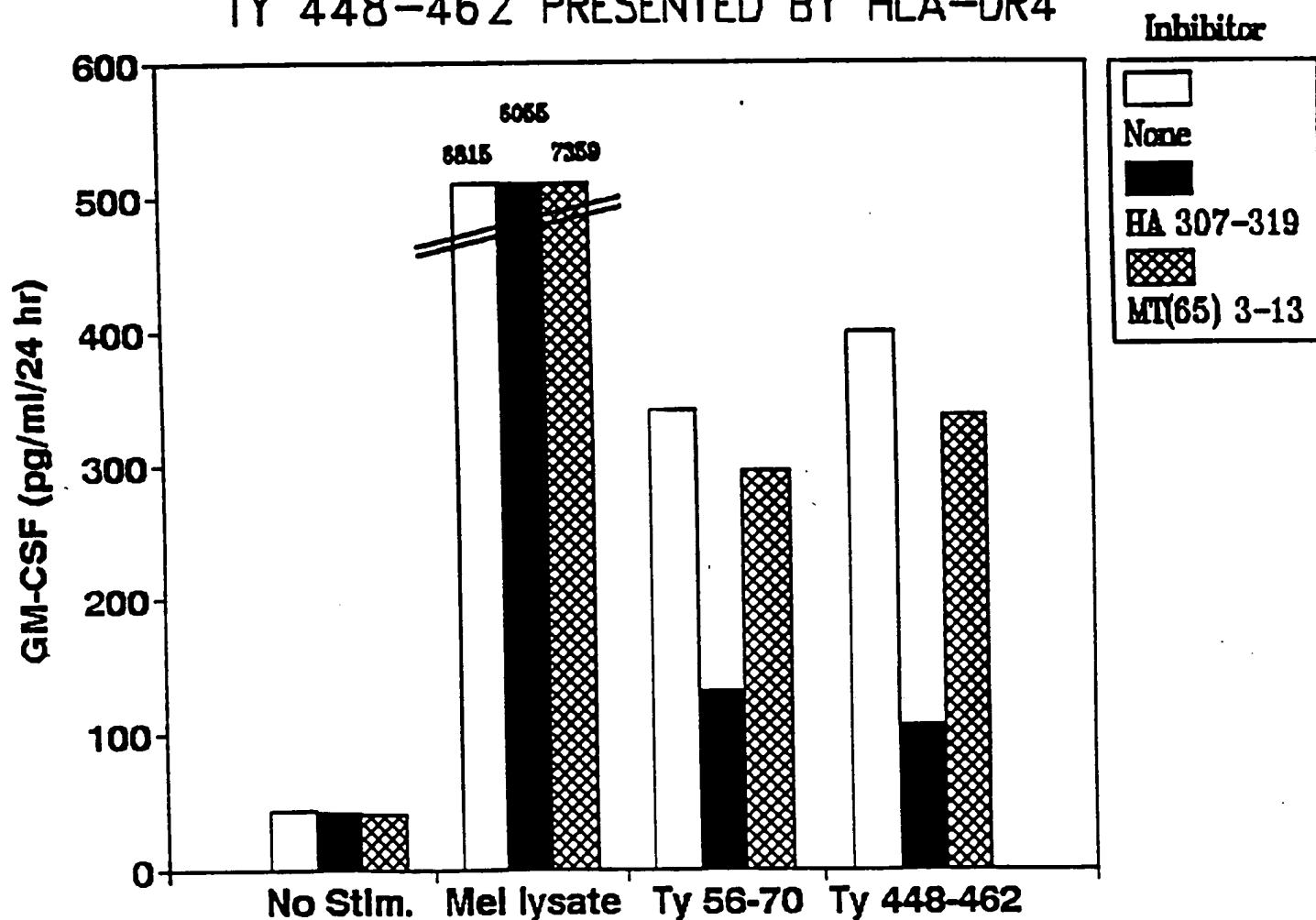


FIGURE 4

HLA Restriction of Tyrosinase Recognition
by CD4⁺ TIL 1088
(GM-CSF secretion, pg/ml/24 hr)

Antigen Presenting Cells	Antigen Presented				HLA-DR β 1*	
	None	1088-mel lysate	Ty 56-70 (A63-V)	Ty 448-462 (D456-V)		
<u>Experiment 1</u>						
1088-EBV	145	1179	2445	2969	0301	0401
697-EBV	282	1014	>500	3815	0401	1501
BM14	751	3282	4793	5121	0401	-
860-EBV	376	870	383	345	0301	1301
583-EBV	484	550	574	477	0404	1501
1087-EBV	507	557	550	566	0701	1201
<u>Experiment 2</u>						
1359-EBV	183	2238	1179	1714	0301	0401
BM14	372	2822	1892	2805	0401	-
1485-EBV	171	160	154	222	0407	1501
COX	544	482	735	703	0301	-
PGF	54	150	76	46	1501	-

Antigen presenting cells pulsed with tumor lysate or 100 μ M peptide overnight,
then washed prior to assay, all cells at 1×10^6 /ml

FIGURE 5

Determining the P1 and P6 Anchor Positions for Ty 56-70

		GM-CSF (pg/ml/24h.)		
		Exp. 1	Exp. 2	Exp. 3
Ty 56-70	Q N I L L S N A P L G P Q F P	295	524	903
I58	- - Q - - - - - - - - - -	<8	<8	<8
	- - F - - - - - - - - - -	39	46	ND
	- - V - - - - - - - - - -	ND	ND	154
L59	- - - Q - - - - - - - - - -	<8	<8	<8
	- - - F - - - - - - - - - -	<8	<8	ND
	- - - V - - - - - - - - - -	ND	ND	<8
L60	- - - - Q - - - - - - - - - -	<8	62	<8
	- - - - F - - - - - - - - - -	86	<8	ND
	- - - - V - - - - - - - - - -	ND	ND	10
A63	- - - - - - - Q - - - - - - -	<8	<8	ND
	- - - - - - - V - - - - - - -	889	2080	ND
P64	- - - - - - - Q - - - - - - -	<8	<8	ND
	- - - - - - - V - - - - - - -	<8	48	ND
L65	- - - - - - - Q - - - - - - -	24	50	ND
	- - - - - - - V - - - - - - -	2050	3816	ND
Ty 57-70	- - - - - - - - - - - - - - -	ND	ND	615
Ty 58-70	- - - - - - - - - - - - - - -	ND	<8	<8
Ty 59-71	- - - - - - - - - - - - - - -	ND	<8	<8
Ty 60-72	- - - - - - - - - - - - - - -	ND	<8	<8
Ty 61-75	- - - - - - - - - - - - - - -	ND	<8	ND
1088mel Lysate		6762	>5500	ND

TIL & EBV backgrounds for Exp.1=53, Exp.2=296, Exp.3=99 pg/ml.
 These have been subtracted from the numbers shown above.

FIGURE 6

OR 533895

Ty 56-70

Q N I L L S N A P L G P Q F P

Ty 448-462

D Y S Y L Q D S D P D S F Q D

FIGURE 7

CD4+ 1088 TIL RECOGNIZE MODIFIED TYROSINASE 56-70 PEPTIDES

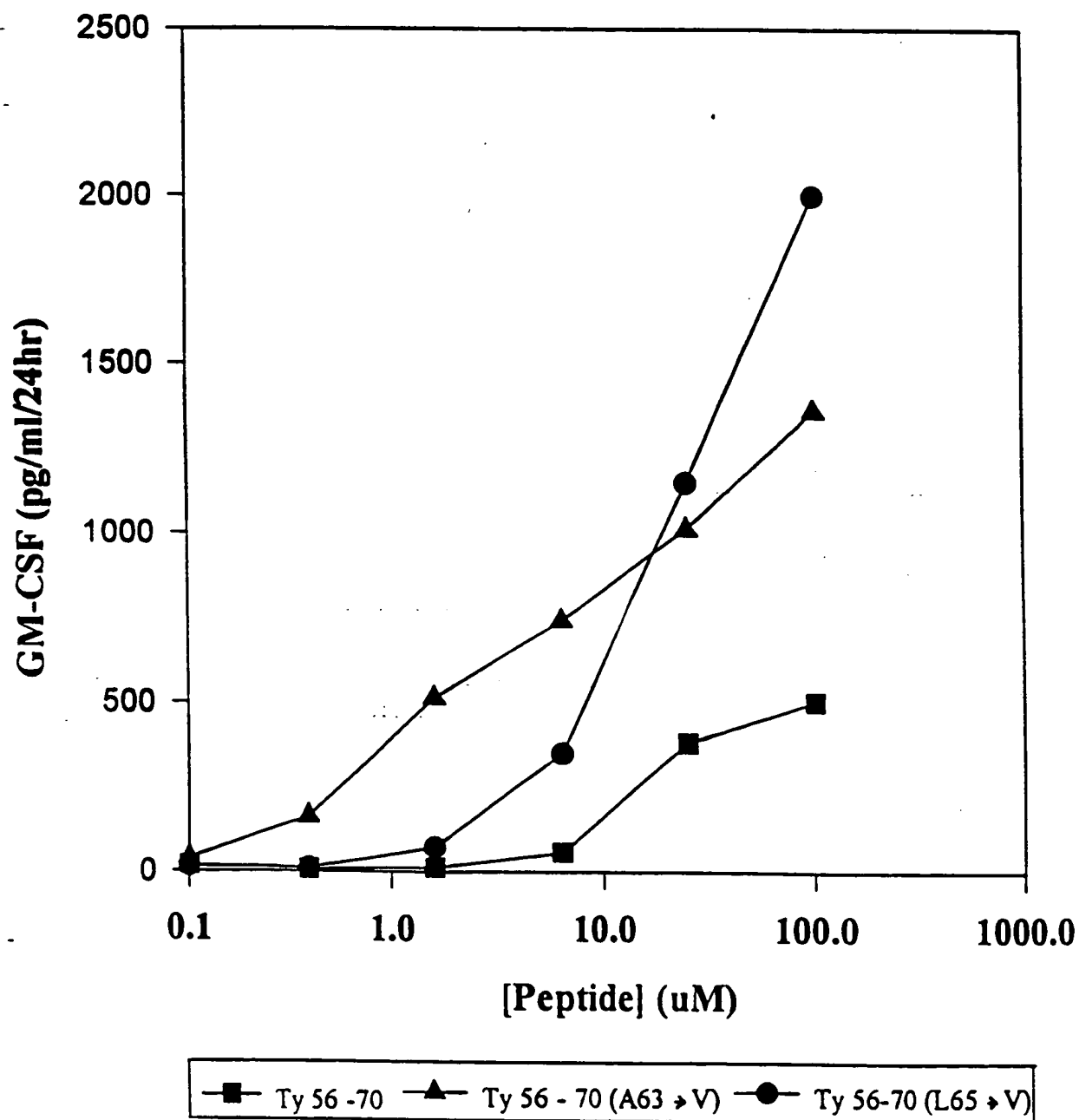


FIGURE 8

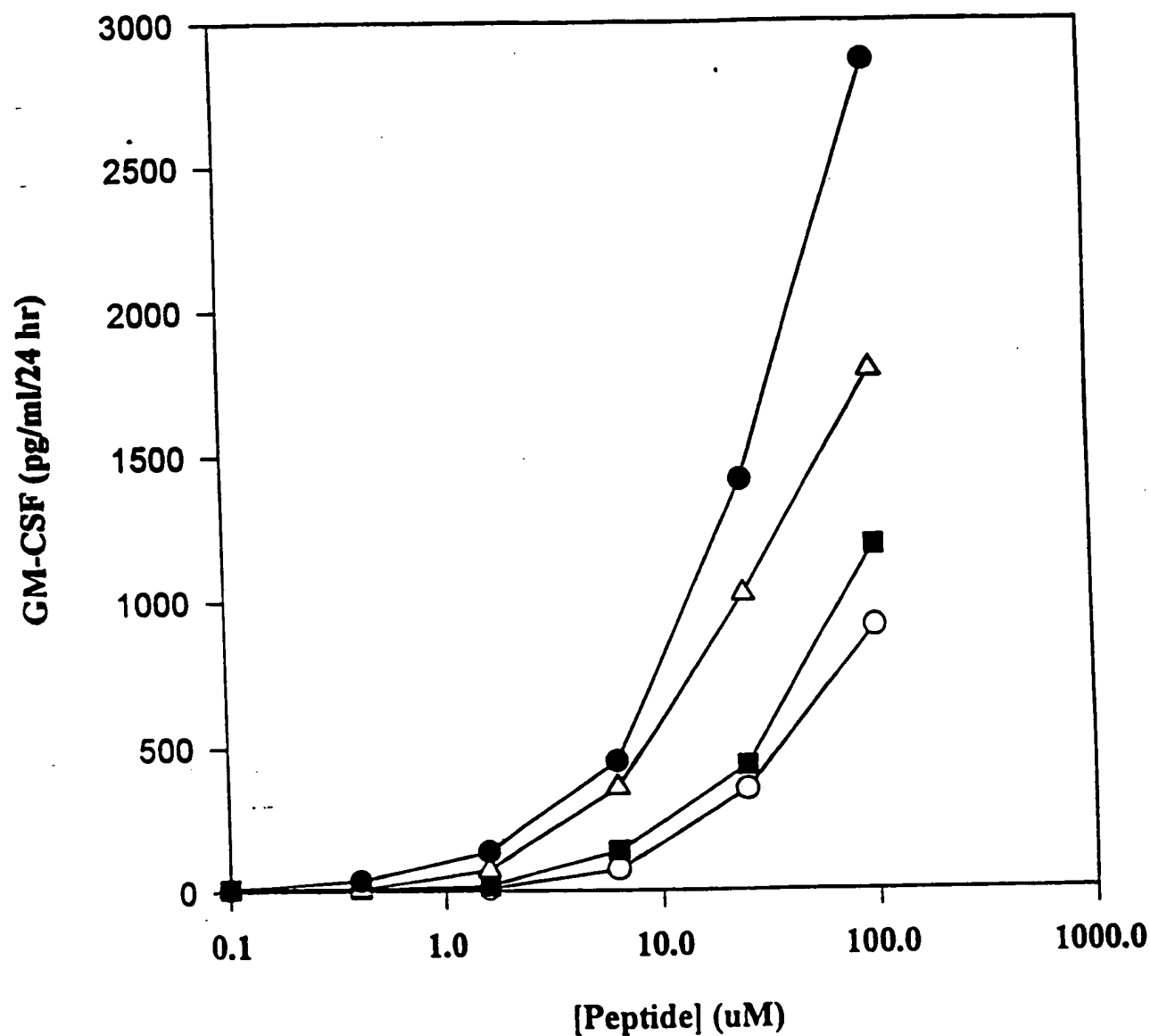
Determining the P1 Anchor for Ty 448-462

		GM-CSF (pg/ml/24h.)	
		Exp. 1	Exp. 2
Ty 448-462	D Y S Y L Q D S D P D S F Q D	108	802
Y449	- Q - - - - - - - - - -	74	520
	- F - - - - - - - - - -	103	781
Y451	- - - Q - - - - - - - - -	28	<8
	- - - F - - - - - - - - -	324	1065
L452	- - - - Q - - - - - - - - -	14	<8
	- - - - F - - - - - - - - -	14	<8
Ty 449-462	- - - - - - - - - - - - -	485	1960
Ty 450-462	- - - - - - - - - - - - -	556	2749
Ty 451-462	- - - - - - - - - - - - -	21	<8
Ty 452-462	- - - - - - - - - - - - -	18	<8
1088-mel Lysate		>5500	5822

TIL & EBV backgrounds for Exp.1=52 and Exp.2=196 pg/ml.
These have been subtracted from the numbers shown above.

FIGURE 9

CD4+ TIL 1088 RECOGNIZE MODIFIED TYROSINASE 448-462 PEPTIDES



—○— Ty 448-462 —■— Ty 448-462 (Y451 → F) —△— Ty 449-462 —●— Ty 450-462

FIGURE 10

CD4+ TIL 1088 Recognize Modified Ty 448-462

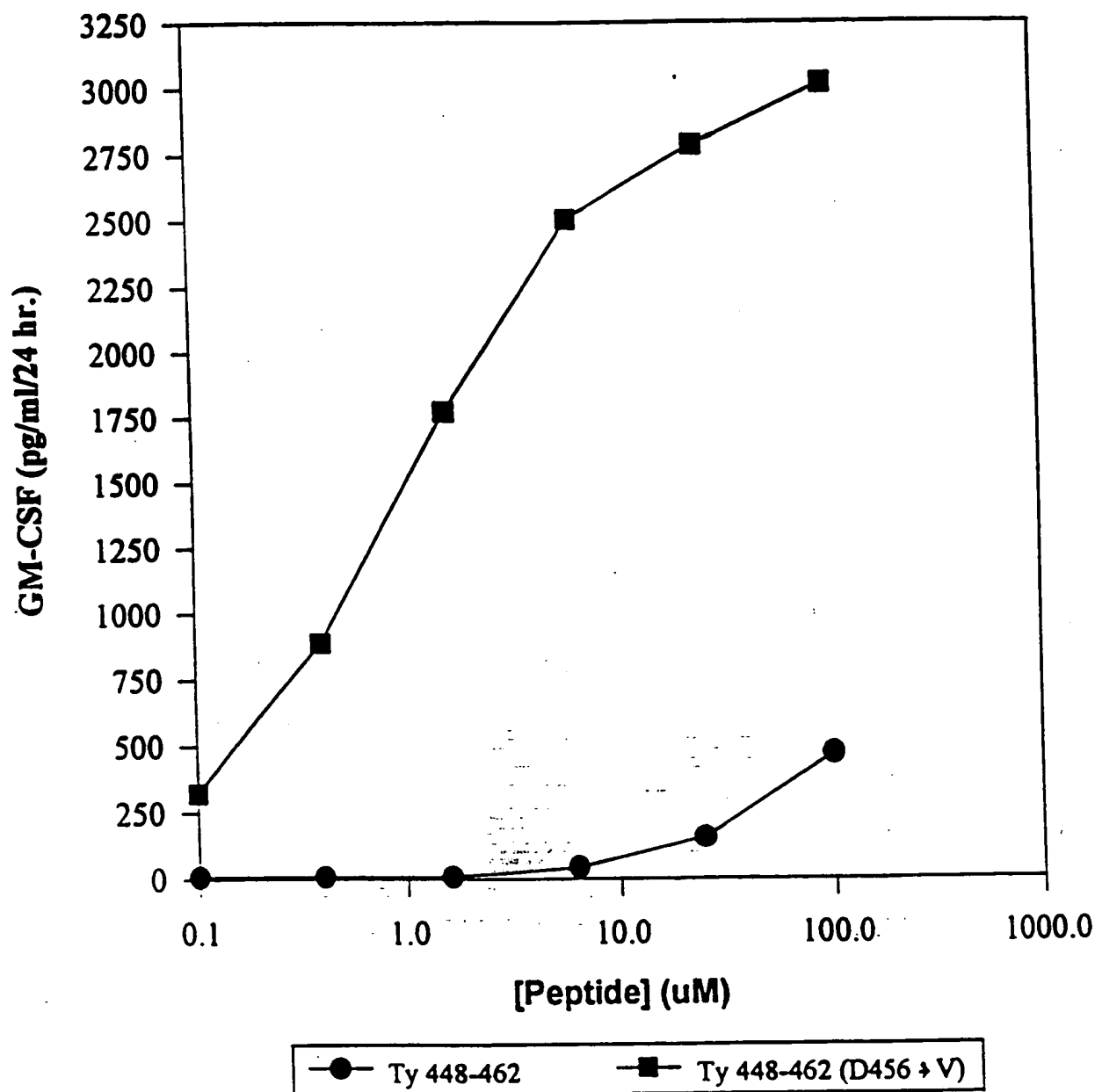


FIGURE 11

CD4+ TIL 1088 RECOGNIZE MODIFIED TYROSINASE 448 - 462 PEPTIDES

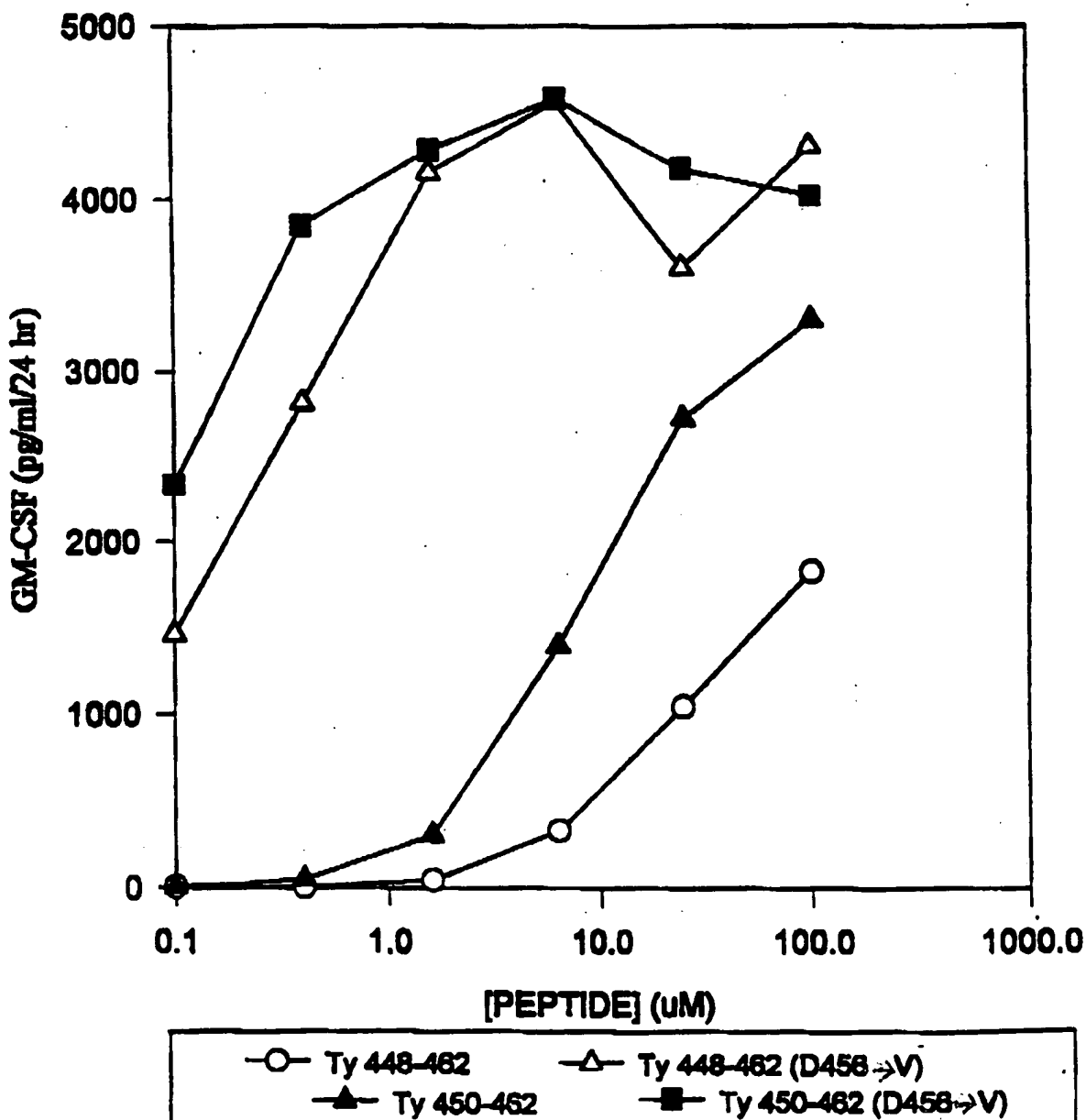


FIGURE 12